Webinar Q&A Report:
Advances in Sub-cellular and Cellular *in vivo* Imaging for Systems Biology

1. **Would it be possible to measure the intensity of the dye/fluorophore in an image as an indicator of drug absorption?**

   Yes, but you would have to collect some reference images to calibrate any variations in sensitivity across the field of view. Any reasonable image analysis software package can then be used to measure intensity as an indicator of drug absorption. There will be some difference between a reference image and artefacts from the real tissue which can be factored as a limitation in the precision of the measurement. However, if a cell is dark and becomes bright, and continues to get, say a further 100% brighter, then this is a real reflection of an increase in fluorophore.

2. **Could you please explain the imaging of colon cancer cells?**

   The View*n*Vivo would be well suited for imaging colon cancer cells. In small animals this must be done surgically. A small incision would be made and the probe inserted to the site of interest. In larger animals, a speculum can be used to insert the probe "non-invasively" and contact with the rectal mucosa can be made directly. As a point of reference, the application note titled "Molecular and Receptor Targeted Imaging" provides an example of how body wall metastases can be visualized using the View*n*Vivo in a rat model of (pancreatic) cancer.

3. **What fluorescent markers would you recommend for staining cells for in vivo microscopy if interested in observing cell cycle life?**

   There are too many to list, however there are several resources available to determine fluorophores that will work for your application. The Optiscan | View*n*Vivo Application Notes are a good starting point. They include a brief protocol and details of the dye used, concentration and usually also the route of administration.

   Another great option for finding suitable markers would be the ThermoFisher Fluorescence SpectraViewer webpage found here: https://www.thermofisher.com/ca/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html

4. Is there any damage to cells as a result of the laser from in vivo confocal microscopy?

Generally, no… as the system is limited to 1mW of excitation, which is safe for extended exposure to most tissues. Also note that the scanning spot is always moving, so each cell only "sees" the laser pass by intermittently. The exceptions are photoactive dyes, which are designed to activate under illumination and cause some biological response. A good example is the use of as 5-aminolevulinic acid (5-ALA), which cells convert to Protoporphyrin IX (PPIX). Most cells do this; however, healthy cells clear the PPIX rapidly whereas certain cancers cannot clear it efficiently. So, the photoactive PPIX is retained in cancer cells after healthy cells are back to a low level. PPIX is fluorescent and can be imaged, but at high laser power and high PPIX concentration, it is activated and leads to blebbing and cell death.

5. How long can you record a fluorescent stain cell?

This is a good question, but there is no simple answer. How long one can record from a fluorescent stained cell depends on the fluorophore and how quickly it bleaches. There are quite a few variables involved in exhausting a fluorophore. These are tissue and fluorophore dependent and can change depending on the intensity of the laser being used. There are very good resources available that discuss bleaching and its minimisation which should be referred to. For example, there are antifade agents that stabilise the fluorophore. Also, good to note is that things like high oxygen environments increase bleaching, so it important not to over oxygenate the tissue or cell medium.

6. A recommendation to use the green laser % in a video microscopy record and avoid cellular damage?

Please refer to the answer to question 4 regarding cellular damage with the View*n*Vivo system. Regarding this question, it is important to appreciate that longer wavelengths produce a lower resolution image, so this particular feature is not available right now with our system.

7. In your opinion, would a transfected cell which can express the GFP protein better tolerate and be more viable when exposed to the laser compared to a cell marked with MitoTracker green?

This really depends on which protein you are co-expressing withy GFP and how concentrated they will be versus the number of molecules that MitoTracker green with label. Either should be fairly robust, but for transfection, consider using eGFP (enhanced GFP) which is brighter and more photostable.

8. Is it possible to work with more than one color?

The system only has one laser in the blue range (488nm). However, the system has standard filters that allow you to spectrally separate the emissions if the spectra from the two fluorophores that you will use are different enough from each other. So, you can capture an image, switch the filter in seconds on the software, and capture the same image with a different filter. This will allow you to overlay the images later in a 3rd party software and colourize them separately. Sometimes you can also get different emissions from one dye depending what they bind to.

9. What is the "working distance" of the optics (depth of imaging)? Can you really image deep from the surface of the optics?

The View*n*Vivo system can image up to 400 microns deep, however, the density and pigmentation of the tissue will limit this distance. We would need to know more about the tissue being measured in order to better answer this question. For example, a tissue such as cornea is normally completely transparent and you would capture a full 400 um depth. Cornea is also permeable to some dyes so you would be able to stain it easily. Compare this to gut epithelium, however, which varies throughout the gut. In the small bowel we see about 200um depth with an intravenous dye such as fluorescein, while with a topical dye like Acriflavine or Proflavine, we might only see superficial epithelial cells because this dye binds avidly to acidic organelles and is usually mopped up and doesn't get far into most tissues. Gastric mucosa is highly scattering and usually we only see about 50um. Generally, speaking, a lot of tissues offer 100-200um image depth.

If you have additional questions for Scintica Instrumentation regarding content from this webinar or wish to receive additional information about the Optiscan | View*n*Vivo Preclinical Imaging System, please contact them by phone or email:

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